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Behaviour of polydiacetylene vesicles under different conditions of temperature, pH and chemical components of milk

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ABSTRACT

Blue polydiacetylene vesicles were studied with regard to their behaviour under variations in storage temperature, heating, potentiometric titration and in the presence of chemical components of milk, to evaluate their application as a sensor in the food industry. Vesicles were prepared using 10,12-pentacosadienoic acid (PCDA)/1,2-dimyristoyl-*sn*-glycero-3-phosphatidylcholine (DMPC). Their changes were monitored using UV–Vis absorption. Temperatures not exceeding 25 °C did not cause colour change in PCDA/DMPC vesicles for a period of up to 60 days of storage. Heating for 10 min at 60 and 90 °C, exposure to pH higher than 9.0 and the simulant solutions of the whey proteins, β-lactoglobulin and α-lactalbumin, promoted colour change from blue to red for the vesicles studied. The effects of routine factors on the characteristics and stability of polydiacetylene vesicles is important in defining the parameters related to their application as a sensor for the food industry.

1. Introduction

The food industry requires quick and satisfactory methods to ensure product safety and process control. An interesting and promising alternative to meet this need is the development of sensors that can be used at any stage of food processing. Some authors have addressed the use and development of sensors and biosensors in the food industry and emphasised their advantages, compared to traditional methods of analysis, being more specific, simple and able to provide quick responses with minimal sample preparation steps (Homola et al., 2002; Mello & Kubota, 2002; Parker & Tothill, 2009).

Materials are being sought that are suitable for the development of sensors and biosensors to be applied in various areas of the food industry. Polydiacetylene (PDA) vesicles have been suggested, because PDA-based materials have different colorimetric characteristics, depending on their environment. Changes in their colour, usually from blue to red, in response to stimuli, such as temperature (Guo, Zhang, Jiang, & Liu, 2007), pH (Cheng, Yamamoto, & Stevens, 2000; Kew & Hall, 2006), mechanical disturbances (Lee, Chae, Ahn, Ahn, & Yeo, 2007), solvents (Yoon, Chae, & Kim, 2007) and recognition of substances (Jung, Park, & Kim, 2006), have promoted the effective use of PDA vesicles in the development of colorimetric analysis (Deng et al., 2009; Kolusheva, Shahal, & Jelinek, 2000; Su, 2005), chips (Kim, Lee et al., 2005; Park, Kang, & Sim, 2008) and biosensors (Lee et al., 2007; Park, Tothill et al., 2008). According to Reppy and Pindzola (2007) the optical properties of PDA vesicles and their susceptibility to their environment are the basis for the generation of signals in PDA-based biosensors. Thus, it is observed that the characteristics of colour change in PDA vesicles, make them suitable as a material for the development of sensors for the food industry.

This study investigated the effect of temperature, pH, and some solutions that simulate the chemical components of milk on colour properties of PCDA/DMPC vesicles, to verify their application in sensors for the food industry. This study focused on the UV–visible spectrophotometric detection of colour change.

2. Material and methods

2.1. Material

Vesicles were prepared using 10,12-pentacosadienoic acid (PCDA) 97.0% (Sigma®); 1,2-dimyristoyl-*sn*-glycero-3-phosphatidylcholine (DMPC) 99.8% (Merck, Darmstadt, Germany) and chloroform HPLC Grade 99.8% (Merck). NaOH ACS reagent 97.0% and HCl ACS reagent 37.0% (both Sigma–Aldrich, St Louis, MO) were used for the titration of vesicles. NaCl 99.95%; $NaH_2PO_4{\cdot}H_2O$

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99.0%; $C_6H_5Na_3O_7{\cdot}2H_2O$ 99.0%; KCl 99.0%; KH_2PO_4 99.0%; $CaHPO_4$ 98.0% and $MgHPO_4{\cdot}3H_2O$ 99.0% (all from VETEC Química Fina Ltda, Rio de Janeiro, Brazil); $CaCl_2$ 99.0% (Merck); $MgCl_2$ 98% (Merck); D-lactose monohydrate, ACS reagent (Sigma); α-lactalbumin from bovine milk 90.0% (Fluka); β-lactoglobulin from bovine milk 90.0% (Sigma) and casein from bovine milk 90.0% (Sigma) were used to simulate the chemical components of milk.

2.2. Preparation of the PCDA/DMPC vesicles

The vesicles were prepared by separately dissolving 10,12-pentacosadienoic acid (PCDA) and 1,2-dimyristoyl-*sn*-glycero-3-phosphatidylcholine (DMPC) in chloroform at a concentration of 1 mM and mixing them, at a ratio of 7:3 (v:v) to a final volume of 10 mL. Chloroform was evaporated using N_2 gas. Then, 10 mL of Milli-Q deionised water (18.1 MΩ resistance) were added. The suspension was heated to 60 °C in a sonicator (Soni-tech ultrasonic cleaner, ultrasonic cleaning HW 800) for 1 h. It was then filtered through polyvinylidene filter (PVDF 0.45 μm, Mille; Millipore Corp., Billerica, MA). The filtrate was cooled to 4 °C for at least 4 h. The vesicles were polymerised by exposure to 254 nm UV light for 15 min.

2.3. Effect of temperature variation on the stability of vesicles

The vesicle suspension was stored at temperatures of 5, 12, 20 and 25 °C for 60 days and monitored by UV–Vis spectrum scanning from 700 to 400 nm (GBC UV/Vis 918; GBC Scientific Equipment, Braeside, Australia), to evaluate the effect of storage time and temperature on vesicle chromism. The spectroscopic analyses were performed on the day the PCDA/DMPC vesicles were produced and after 7, 15, 22, 30 and 60 days. The same spectrum scanning was made to evaluate the thermochromism of the PCDA/DMPC vesicles with heating at 30, 60 and 90 °C for 10 min.

2.4. Effect of pH on the stability of vesicles

The vesicle suspension was titrated potentiometrically with NaOH (0.1 M, pH 9.8) and the pH readings were carried out after a 5 min with a potentiometer (Digmed DM20), and simultaneously monitored by UV–Vis spectrum scanning from 700 to 400 nm, to evaluate the effect of pH on the chromic phase transition of the vesicles. HCl (0.1 M, pH 0.98) was also used to assess chromic response at pH values <4.0. The analyses were performed at 21 ± 2 °C.

2.5. Effect of the chemical components of milk on the stability of vesicles

Solutions that simulate the concentration of some components of milk were added individually to the PCDA/DMPC vesicle suspension according to Table 1. The effect of each solution individually on vesicle chromism was monitored by UV–Vis scanning from 700 to 400 nm; at first, 5 min after the addition of solutions of the simulants; next, at intervals of two or four days for a period of 12 days, at 21 ± 2 °C. In the same away we also evaluated the effect of fat, obtained by centrifugation of raw milk, according to the method suggested by R-Biopharm Rhône Ltd., and direct addition of UHT milk.

The concentrations of the solutions that simulated the components of milk were generally prepared according to the theoretical concentrations (total average) suggested by Walstra, Wouters, and Geurts (2006): carbohydrates–lactose (4.9%); salt–Na (48 mg/100 g), K (143 mg/100 g), Ca (117 mg/100 g), Mg (11 mg/100 g), citrate (175 mg/100 g), proteins–casein (26 g/kg), β-lactoglobulin (3.2 g/kg) and α-lactalbumin (1.2 g/kg).

Table 1
Concentration of solutions of milk components added to PCDA/DMPC vesicle suspension.

Component	Concentration (wt.%)	Final pH
NaCl (sodium chloride)	0.122	6.3
NaH_2PO_4 (sodium phosphate)	0.288	6.0
$C_6H_5Na_3O_7$ (sodium citrate)	0.275	7.3
KCl (potassium chloride)	0.275	6.0
KH_2PO_4 (monopotassium phosphate)	0.503	6.0
$CaCl_2$ (calcium chloride)	0.337	6.6
$CaHPO_4$ (dicalcium phosphate)	0.405	7.0
$MgCl_2$ (magnesium chloride)	0.44	6.8
$MgHPO_4$ (magnesium phosphate).	0.080	7.0
Lactose	5.0	6.2
Fat	3.0	–
	Concentration (g/L)	
α-Lactoalbumin	1.5	–
β-Lactoglobulin	4	–
Casein	28	–

2.6. Colorimetric response

In cases of colour change, from blue to red, the colorimetric response (CR) was calculated as a semi-quantitative parameter of the change of chromic properties, according to the following equation (Okada, Peng, Spevak, & Charych, 1998):

$$\mathrm{CR}(\%) = 100 \times \frac{B_o - B_1}{B_o} \quad (1)$$

where $B = (A_{blue}/(A_{blue} + A_{red}))$; A_{blue} = absorbance at 640 nm and A_{red} = absorbance at 540 nm; B_o and B_i values calculated before and after colour change, respectively.

For all tests, a descriptive analysis was carried out for the behaviour of the samples. The experiments were prepared with at least three replicates.

3. Results and discussion

3.1. Effect of temperature variation on the stability of the vesicles

The PCDA/DMPC vesicles presented no colour transition, no aggregates formation and the same behaviour (spectrum indicative of the blue-phase PDA with an absorption maximum at ≈635 nm) when subjected to temperatures of 5, 12, 20 and 25 °C for a period of 60 days. However, storage at temperatures of 20 and 25 °C for 60 days led to change in the vesicles' colour intensity, with absorbance values of approximately half those of their initial value (time 0). Possible changes in the vesicle structure, which were not sufficient to change colour from blue to red, promoted the decrease in blue colour intensity at 20 and 25 °C. These data indicate that the

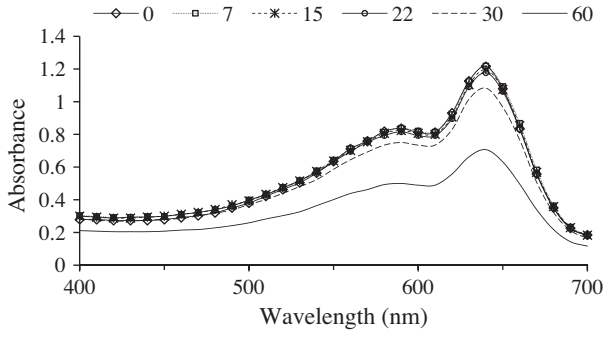

Fig. 1. Absorption spectra for PCDA/DMPC vesicles at 25 °C storage. 0, 7, 15, 22, 30 and 60 refer to the time of storage in days.

vesicles were stable for 60 days under storage at 5 and 12 °C. Fig. 1 represents the absorption spectrum obtained for storage at 25 °C to illustrate the behaviour exhibited by the vesicles during this evaluation. The spectrum presented here is different from that presented for the storage at 5 and 12 °C only on the last day and is similar to the spectrum presented for the storage at 20 °C during all the periods studied. Some authors declare that PDA vesicles can be stored under refrigeration temperatures for a long period of time without losing their characteristics (Pevzner, Kolusheva, Orynbayeva, & Jelinek, 2008; Schimitt, 2003). Okada et al. (1998) developed vesicles that remained stable for a long time and did not present evidence of melting or formation of large aggregates once polymerised. In our studies, storage at temperatures lower than 20 °C for a period of 60 days maintained the stability of PCDA/DMPC vesicles and no aggregates were observed.

However, when the vesicles were subjected to heating at temperatures of 30, 60 and 90 °C for 10 min, a colour transition was thermally induced, whereas heating at 30 °C resulted in no thermochromism. Fig. 2 shows the spectrum obtained with colour change at the heating temperatures mentioned. With increasing temperature, intensity of absorbance at the range of 630–640 nm (blue phase) became smaller, while intensity at the range of 530–540 nm (red phase) became larger, which indicates a change in the range of absorption in the visible spectrum by the vesicles. This behaviour indicates that warming caused irreversible changes in the chromic characteristics of PCDA from blue to red. Quantification by colorimetric response indicated values of 10.78% and 68.86% at 60 and 90 °C, respectively.

Colour transition due to heating was observed in PCDA vesicles in various situations. Several authors have found irreversible colour transition from blue to red, which agrees with the findings of our studies when heating PDCDA/DMPC vesicles at temperatures of 30, 60 and 90 °C for 10 min. Kim, Lee, Choi, Sonh, and Ahn (2005) monitored colour change by UV–Vis spectroscopy, for PCDA vesicle suspension after gradual warming to 90 °C and reducing temperature to 25 °C, and observed irreversible colour transition of the vesicles. Lee, Chae et al. (2007), found colour transition for PCDA vesicles dispersed in a solution consisting of poly-vinyl alcohol and sodium borate at temperatures from 40 to 55 °C, with CR of 30% at 55 °C. In studies carried out by Potisatityuenyong, Tumcharern, Dubas, and Sukwattanasinitt (2006), PCDA vesicles in solution presented complete colorimetric transition at the range of 68 °C and CR ranging from 20% to 70%. These values were linear for temperatures between 25 and 70 °C. Vesicles composed of PCDA and various amino acids and also underwent colorimetric transition due to the effect of heat treatment and the thermal sensitivity varied according to the amino acids added to the vesicles. It was highest for vesicles composed of glutamine/PCDA (Cheng et al., 2000). Whereas the effect of temperature is related to the change in the PDA structure from planar form to nonplanar form (Guo et al., 2007), these studies indicate that warming leads to changes in PCDA structure, and the addition of other components to the composition of PCDA vesicles may affect their thermal sensitivity.

The results obtained suggest that colour transition in PCDA/DMPC vesicles, from blue to red, can be used for the development of sensors to be used in the food industry to monitor temperature variations at different stages of processing.

3.2. Effect of pH on the stability of PCDA/DMPC vesicles

Another important stimulus that is known to cause colour change in PDAs is the pH variation. The spectrophotometric results obtained by the addition of 0.1 M NaOH to the PCDA/DMPC aqueous vesicle suspension (initial pH 6.2 and pH values of 7.3, 8.2, 8.9, 9.1, 10.0, 11.0 and 12.2 obtained after NaOH addition) are shown in Fig. 3. The NaOH titration provided colour transition from blue (maximum absorption 640 nm) to red (maximum absorption 540 nm) in vesicles at pH above 9.0 and the formation of intermediate chromic phase was not observed. The colorimetric response (CR) values were 26%, 44%, 38% and 33% at pH 9.1, 10.0, 11.0 and 12.2, respectively. Colorimetric response values $\geqslant$15% are visible to the naked eye (Boullanger et al., 2008). On the other hand, the addition of 0.1 M HCl (to give pH values of 5.4, 5.0, 3.5, 3.0 and 2.5) and acidification of the vesicles at pH values lower than 4.0 provided no change in the colorimetric properties of vesicles (there was no change in colour), but led to the formation of aggregates of vesicles and turbidity in the medium, which prevented spectrophotometric measurements. The results are similar to those presented by Kew and Hall (2006), for 10,12-tricosadienoic acid vesicles. These authors observed irreversible colour change from blue to red when pH was increased by adding NaOH and the formation of precipitate at pH below 4.0. They also observed the formation of an isosbestic point, indicating that the colour change from red to blue occurred without formation of intermediate colour. The same can be seen in Fig. 3 for the PCDA/DMPC vesicles studied except at pH 12.2. In this case the pH value promoted the colour change from blue to red without formation of intermediary colour and also promoted changes in the vesicle structure that caused decrease in red colour intensity, with absorbance values of approximately half those of the others.

In these studies, the effects that lead to colour change due to variation in pH were not evaluated, but some authors have suggested mechanisms to elucidate such chromatic transitions. Song, Cheng, Kopta, and Stevens (2001), suggested that colour change from blue to red is caused by increased electrostatic repulsion

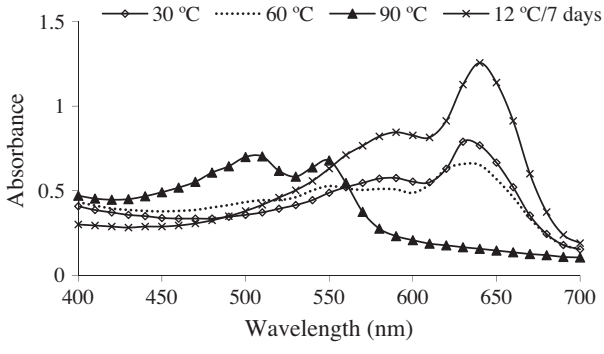

Fig. 2. Absorption spectra for PCDA/DMPC vesicles heated for 10 min at 30, 60 and 90 °C, compared to the reference (vesicle suspension without heating stored at 12 °C/7 days).

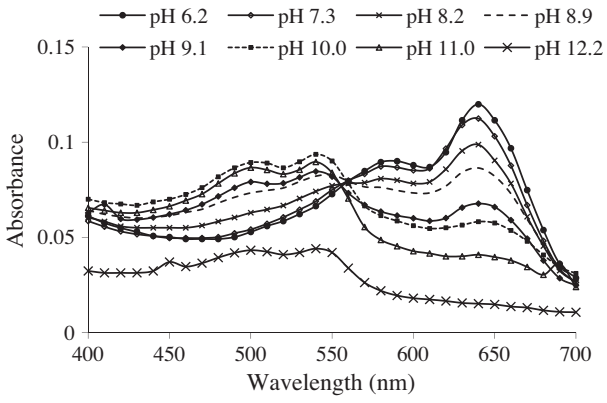

Fig. 3. Spectral curves representing the absorbance for the PCDA/DMPC vesicle solution after potentiometric titration using NaOH (0.1 M).

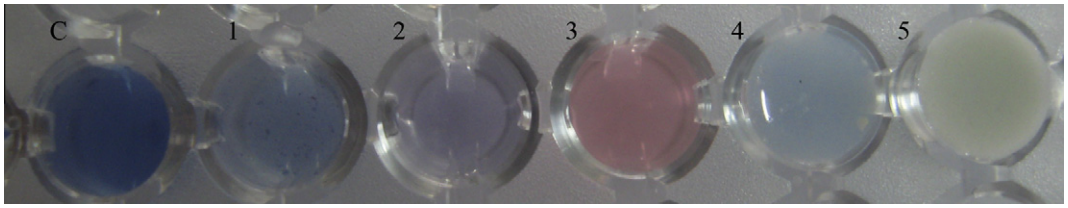

Fig. 4. Effect of milk component solutions on PCDA/DMPC vesicles after 12 days of storage. (C) Control; (1) colour after the addition of solutions containing $CaCl_2$, $CaHPO_4$, $MgCl_2$, $MgHPO_4$; (2) intermediate violet colour; (3) colour transition presented by the addition of α-lactoalbumin and β-lactoglobulin; (4) vesicles after the addition of milk; (5) vesicles after the addition of fat. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

among the head groups, due to elevation of pH caused by adding NaOH. Kew and Hall (2006) proposed that the change in colour due to pH is related to Coulomb repulsion among head groups, which can cause conformational disturbances in PDA structure. Boullanger et al. (2008) suggested that increased pH leads to ionisation of PDA carboxyl groups, which induces some twists in polymer structure, thus reducing the activation energy required for colour change to begin.

The results obtained for the effect of pH on the stability of PCDA/DMPC vesicles suggest that the conditions that expose vesicles to pH values above 9.0 can cause changes in the colorimetric properties of vesicles, while pH values lower than 4.0 may promote destabilisation of the vesicles. Hence, vesicles with this composition can be used to develop sensors for the food industry without losing their chromic characteristics in situations in which pH values range from 5.0 to 8.0. The PCDA/DMPC vesicles can also be used as colorimetric tests, indicating change of pH in systems with pH above 8.0.

3.3. Effect of chemical components of milk on the stability of vesicles

PCDA/DMPC vesicles have potential to be used in the dairy industry. For example, they could be used as a tool to develop biosensors for the detection of aflatoxins in milk and dairy products (this project has been developed by the Universidade Federal de Viçosa packaging laboratory). The effect of some milk components on the chromic properties of vesicles was studied to assess the possible use of PCDA/DMPC vesicles in dairy products.

The assessment in the UV–Vis region of the vesicles after the addition of salt solutions at a ratio of 1:1 (v:v) and storage at room temperature of 21 ± 2 °C revealed that the addition of solutions of $CaCl_2$, $CaHPO_4$, $MgCl_2$ and $MgHPO_4$ induced the formation of precipitates of vesicles from the 4th day of storage, but colour transition from blue to red was not observed in any case. The vesicles that received these solutions showed decreased absorbance values (for wavelength ranging from 630 to 640 nm) over time, indicating that the formation of the aggregates affects the intensity of the blue colour initially presented by the vesicles. The other salt solutions and the lactose solution, the addition of casein, fat or UHT milk did not cause changes in the characteristics of blue colour of the PCDA/DMPC vesicles studied. The suspensions of whey proteins, β-lactoglobulin and α-lactolbumin led to colour change, from blue to red from the 12th day of assessment, with CR of 39.58% and 27.38%, respectively, indicating that the suspensions of these proteins studied can cause disturbances that lead to chromic transitions of PCDA/DMPC vesicles. Fig. 4 illustrates the aspect presented by the PCDA/DMPC vesicles after the addition of milk component simulant solutions and 12 days of storage.

Jose and König (2009) observed that the addition of salts of Na^+, K^+, Ca^{2+} and Mg^{2+} did not affect the chromic characteristics of vesicles composed of PCDA and compounds that bind to metals. Reppy and Pindzola (2007) studied the effect of the addition of divalent and monovalent cation salts on liposomes of poly (10,12-PCDA), poly (6,8-DCDA) and poly (10,12-P-EtOH) prepared in deionised water, and observed that all formulations of liposomes aggregated quickly when exposed to $MgCl_2$, $MnCl_2$ and $CaCl_2$ at concentrations higher than 1 mM, and more slowly when exposed to lower concentrations. In the presence of monovalent salts, they were more stable, but were still clustered in solutions of 200 mM of NaCl and KCl after 1 h. Parker and Tothill (2009) developed an immunosensor for aflatoxin M_1 in milk. They investigated the effect of milk components and observed that milk affected significantly the functioning of the immunosensor and that milk proteins were a major cause of such interference. Since most sensors in the food industry need to be in contact with food components, these studies indicate that the interference of the food matrix on the characteristics of chromic transition and stability of PDA vesicles should always be evaluated for each type of vesicle developed.

4. Conclusions

Temperatures lower than 20 °C can be used to store PCDA/DMPC vesicles for a period of 60 days without destabilising them. Heating for 10 min at temperature of 30 °C does not change the blue characteristics of the vesicle studied while exposure to the same conditions at 60 and 90 °C favours irreversible colour transition of the vesicles from blue to red.

PCDA/DMPC vesicles can be used in food industry without changes in their chromic properties, at pH values ranging from 5.0 to 8.0.

Under the conditions studied, the simulant solutions of the salts $CaCl_2$, $CaHPO_4$, $MgCl_2$ and $MgHPO_4$ favoured the formation of aggregates of vesicles from the fourth day of storage and suspensions of the proteins β-lactoglobulin and α-lactalbumin led to colour transition, from blue to red, from the 12th day of storage.

Knowledge on the effect of the addition of food components to PCDA/DMPC vesicles and their effect on their stability is important to define the parameters relating to their application in the development of sensors for the food industry.

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